XII RBMP

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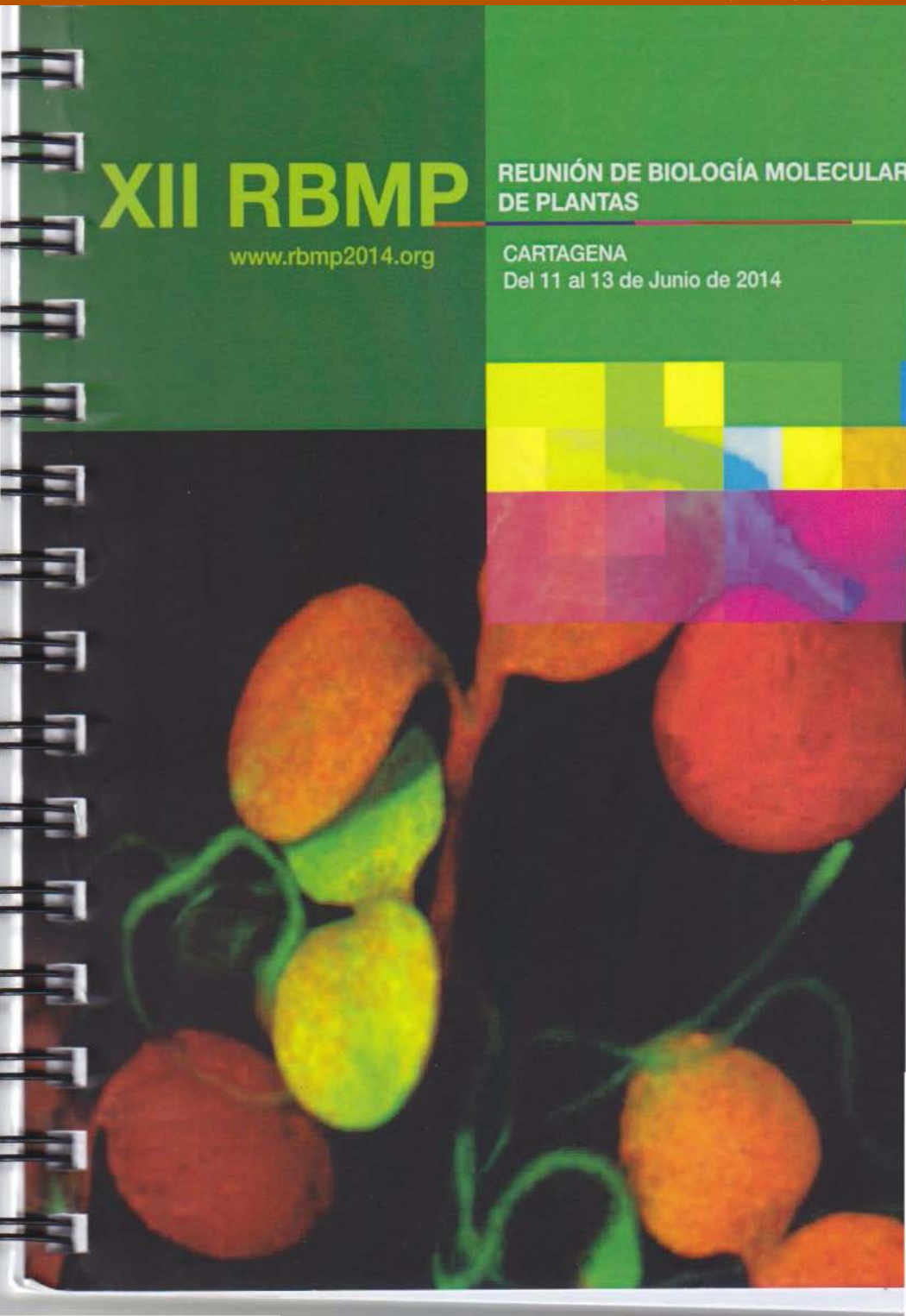

PO 65

The ectopic overexpression of HaHSFA9 induces photo-morphogenesis by enhancing the expression of genes involved in the biogenesis of the photosynthetic apparatus

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We inferred that genes activated by HaHSFA9 (A9) contribute to protection of proplastids from developmental desiccation in sunflower (*Helianthuus annuus*, L.) seeds (1). Here, we report potential target genes of A9 that could explain such protection, which would be coupled to an enhanced photo-morphogenesis in the germinating seed. Using SSH (Subtractive Suppressive Hybridization) we have cloned cDNA for mRNAs transcribed from genes (directly or indirectly) enhanced by A9 in 35S:A9 transgenic tobacco plants. Among the SSH clones for which a putative functional assignment was possible from sequence data, we noted a strikingly high proportion (35-40%) of genes involved in the photosynthetic apparatus. This includes components of the two photosystems (PSI and PSII), the plastidial ATPase complex, the cytochrome b_6f complex, and other genes involved in the biogenesis of chloroplasts. RT-qPCR experiments showed that A9 caused a subtle but wide-ranging enhancing effect of A9 on genes with functions connected to structure and assembly of photosynthetic membranes. Thus, A9 enhanced genes encoding components of the four major thylakoid complexes involved in electron transfer and energy transduction in photosynthetic membranes of chloroplasts: PSII (PsbP, PsbR), cytochrome b_6f (PetM), ATP-synthase (AtpG), and PSI (PsaG, PsaH, PsaN y PsaO). A9 also induced POR1, which encodes a *NADPH:protochlorophyllide oxidoreductase,* an enzyme that catalyzes the sole light-dependent step of chlorophyll biosynthesis. These results suggested that A9 might induce a photo-morphogenic effect. This has been experimentally confirmed by observing changes consistent with such an effect when 35S:A9 seedlings germinated and kept fro 10 days under dark turn green when exposed to light. For example, both the chlorophyllide content (produced in the step catalyzed by POR1) and the total chlorophyll content showed a significantly higher increase in the 35S:A9 seedlings compared to non-transgenic (NT) sibling seedlings. In addition, the etiolated 35S:A9 seedlings have shorter hypocotyls than the NT siblings. Furthermore, after exposure to light the 35S:A9 seedlings expand their cotyledons at a higher rate than the NT siblings. These effects of A9 would be unprecedented for a plant HSF. A9 would thus induce both protective and photo-morphogenic effects in the 35S:A9 seedlings. Such a combined action would help explaining the survival and recovery of the photosynthetic apparatus that was observed in the 35S:A9 seedlings after drastic stress conditions (1), or after other lethal treatments that induce leaf senescence.

(1) Almoguera *et. al.*, PLoS ONE 7: e51443 (2012)

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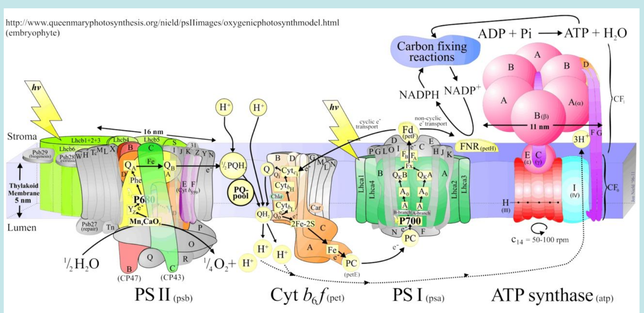
XII Reunión Biología Molecular de Plantas

BACKGROUND AND CONCLUSSIONS

We proposed that genes activated by HaHSFA9 (A9) contribute to protection of proplastids from developmental desiccation in sunflower (*Helianthus annuus*, L.) seeds (1). Using SSH (Subtractive Suppressive Hybridization) we have cloned cDNA for mRNAs transcribed from genes (directly or indirectly) enhanced by A9 in 35S:A9 transgenic tobacco plants, which display gain-of-function for A9 (1, 2). Among the obtained SSH clones we noted a strikingly high proportion (35-40%) of genes involved in the photosynthetic apparatus. This includes components of the two photosystems (PSI and PSII), the plastidial ATPase complex, the cytochrome b_6f complex, and other genes involved in the biogenesis of chloroplasts. RT-qPCR experiments showed that A9 caused a subtle but wide-ranging enhancing effect of A9 on genes with functions connected to structure and assembly of photosynthetic membranes. A9 also induced POR1, which encodes a *NADPH:protochlorophyllide oxidoreductase,* an enzyme that catalyzes the sole light-dependent step of chlorophyll biosynthesis **(Figure 1)**. These results suggest that A9 might induce a photo-morphogenic effect, which has been confirmed (**Figures 2 and 3**). A9, a Heat Shock transcription Factor would thus induce both protective and photo-morphogenic effects in the 35S:A9 seedlings. Such a combined action would help explaining the survival and recovery of the photosynthetic apparatus that was observed in the 35S:A9 seedlings after drastic stress conditions (1), or after other lethal treatments that induce leaf senescence (3).



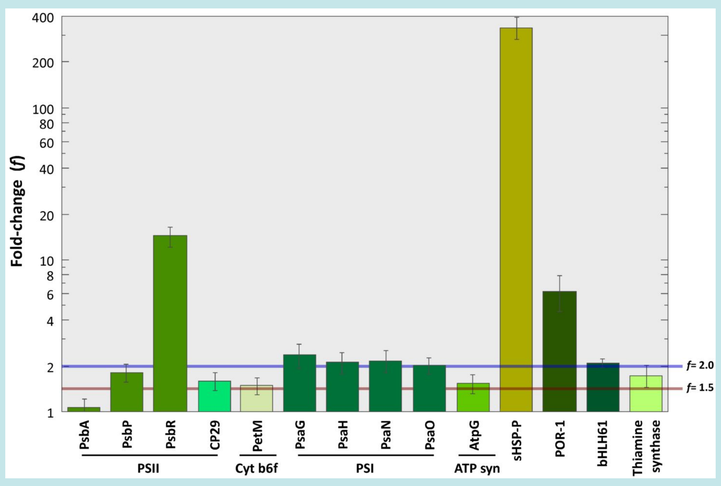


FIGURE 1. Comparison by RT-qPCR of the fold-change between 35S:A9$_1$ and NT$_1$ seedlings for mRNAs of selected SSH clones. The cartoon on top depicts the four major complexes of the photosynthetic apparatus. The normalized average fold-change (f) is represented as the ratio of the mRNA abundance for each gene compared. The amplified cDNAs were obtained from 3-4 week-old seedlings. Average values were obtained from 3 biological replicas for each seedling type (35S:A9$_1$ and NT$_1$), with 2-3 technical repeats per replica. Three genes with unchanged mRNA accumulation in the seedlings (L25, Ef1a y UBc2) were used to normalize gene expression values. The red and blue lines indicate fold-changes above f= 1.5 and f= 2.0, respectively. Among the genes induced by HaHSFA9 in the 35S:A9$_1$ seedlings, we include data for two that are not directly involved in the photosynthetic apparatus: the bHLH61 transcription factor and thiamine synthase. As a positive control we used sHSP-P, which encodes a chloroplast-localized protein with was highly induced in the 35S:A9 seedlings (1). As a negative control we used the chloroplast genome-encoded PsbA (D1) protein.

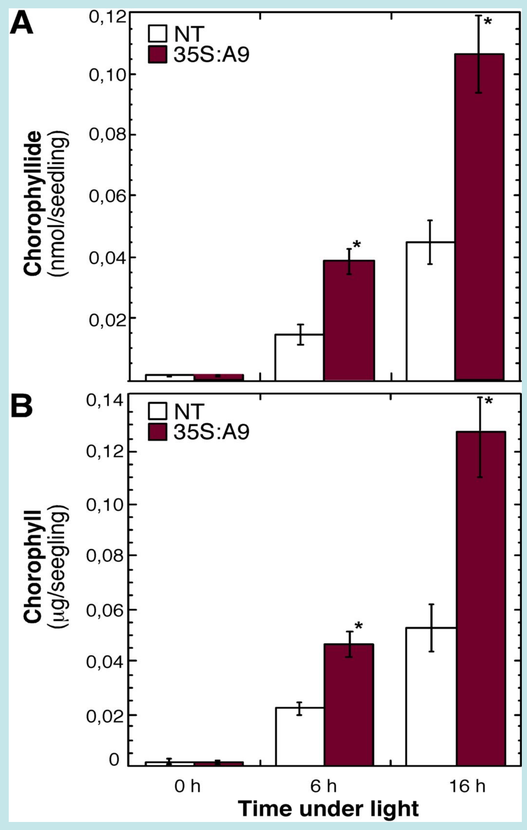


FIGURE 2. Photo-morphogenic effect of HaHSFA9 (I). Higher increase of photosynthetic pigment content in the 35S:A9 seedlings compared to sibling NT seedlings. Tobacco seedlings were germinated and kept in darkness for 10 days (0 h samples), and then were transferred under continuous culture illumination conditions for 6 h or 16 h. (A). Chlorophyllide content (Chlorophyllide is a precursor of chlorophyll synthesized upon exposure to light in a step catalyzed by POR1). (B). Total chlorophyll content. We represent average values from 3 independent experiments performed with seedlings from 3 pairs of sibling 35S:A9 and NT lines (n= 27, per line and condition). Upon exposure to light, statistically significant higher chlorophyllide and total chlorophyll content was observed in the 35S:A9 seedlings, compared to NT (*, P≤ 0.01). These results are consistent with the induction of the POR1 gene by HaHSFA9 in the 35S:A9 seedlings (see Figure 1).

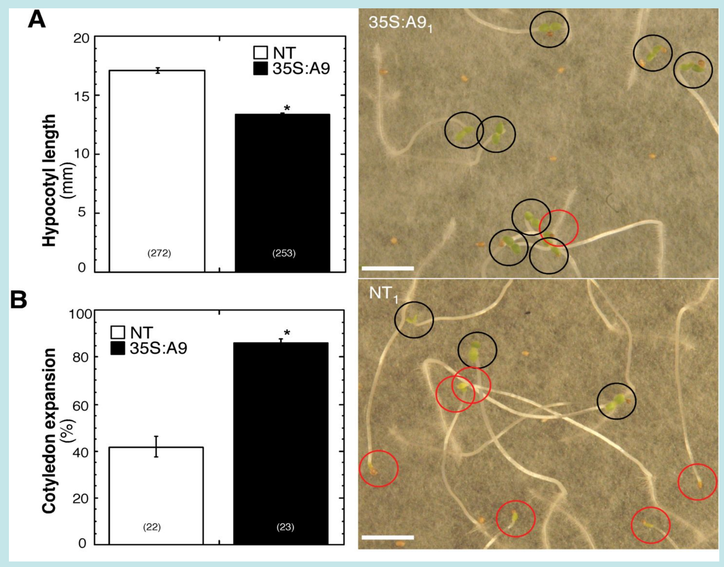


FIGURE 3. Photo-morphogenic effect of HaHSFA9 (II). Differences in post-germinative growth and development between the 35S:HaHSFA9 (35S:A9) and sibling non-transgenic (NT) seedlings. (A). Etiolated 35S:A9 seedlings have shorter hypocotyls than the NT siblings. Hypocotyl length measured 10 days after germination and growth in total darkness. (B). The 35S:A9 seedlins expand their cotyledons at a higher rate than the NT siblings. Cotyledon expansion measured after the etiolated seedlings were exposed to continuous culture illumination for 16 h. We represent average values from 3 independent experiments performed with seedlings from 3 pairs of sibling 35S:A9 and NT lines. Asterisks denote statistically significant differences (*, P≤ 0.01). Numbers in brackets indicate sample sizes. On the right, we show representative pictures for results of cotyledon expansion in one of the experiments performed with the 35S:A9$_1$ and NT$_1$ line pair. Black circles mark seedlings with expanded cotyledons and red circles those with non-expanded cotyledons. Scale bars, 0.5 cm.

FUTURE RESEARCH

We will try to confirm the involvement of A9 in photomorphogenesis by loss-off-function. DS10:A9-SRDX plants (4) could be used to analyze gene expression (and other phenotypes) very early during germination.

Does A9 (HSFA9) enhance photomorphogenesis in other plants? We will try to answer this question. There might be, however, crucial differences in HSF seed gene regulation between Asterids plants (as sunflower) and other dicots including Rosids plants as Arabidopsis (see our second in this meeting: Poster X).
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Acknowledgements

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